TOPIC 10 – Microparticles

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0289

Circulating microparticle levels and vascular function in a mouse model of combined intermittent hypoxia and high-fat diet

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Obstructive sleep apnea-hypopnea syndrome (OSAHS) is a common disease which causes a tendency to fall a daytime sleep, cognitive impairment and decreased a quality of life. Metabolic syndrome (MS) is defined by the association of morphological, physiological and biochemical abnormalities predisposing the affected individual to very high risk of atherosclerosis and cardiovascular pathology. Microparticles (MPs) are small membrane vesicles, released into the extracellular space following to cell activation or apoptosis. Although OSAHS and MS patients display endothelial dysfunction, circulating levels of MPs are either not modified or increased, respectively. Here, we evaluated the effects of the combination of both intermittent hypoxia (IH) and obesity in mice. For this, mice were divided in four groups: (i) control (normoxia and normal diet), (ii) high-fat diet (HFD, 42% calories from fat for 8 weeks), (iii) IH (for 2 weeks, 8 hours of IH/day, 1 stimulus by 30-60 seconds, 60-120 desaturation/hour) and (iv) group receiving both HFD and IH (for 8 weeks, the last two receiving IH). We showed that although HFD did not induce changes in the weight of the mice, it was able to induce early-onset obesity as reflecting by the increase of adipose tissue weight. This was accompanied by an increased rate of macrophage and erythrocyte MPs as well as a decrease in the endothelium-dependent relaxation evoked by acetylcholine suggesting that HFD induces an endothelial dysfunction. On the other hand, exposure to IH induced an increase in the rate of leukocyte, macrophage and red blood cell MPs, without changes in endothelium-dependent relaxation. Finally, animals from the group with both treatments displayed a similar profile of MPs as those treated with IH alone. In addition, IH prevented both fat deposition and endothelial dysfunction induced by HFD alone. These results suggest that IH might protect vascular function in HFD models under these experimental conditions.

0324

The relative charge of circulating microparticules in heme determines their toxicity for endothelial cells

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Rational: Hemolytic disorders like sickle cell disease are linked to the occurrence plasma heme, called 'free' heme. We hypothesized that free heme associates with cell fragments called microparticules (MP) that express phosphatidylserine (PS) at their surface. We thought that free heme associated to MP may prove particularly toxic for vascular'endothelium.

Material and Methods: Sickle cell and healthy erythrocytes were separated from platelet-free plasma (PFP) by Ficoll gradients centrifugation. We purified different types of MP : 1/ MP from PPP, 2/MP shed by erythrocytes in vitro, 3/synthetic MP generated from a PS:Phosphatidylcholine mix, loaded with heme in the presence of calcium. We analyzed their contents in heme by spectrophotometry (540 nm) and Western blot, and characterized MP par FACS after labeling with annexin-V. We compared the effects of the purified MP on endothelial monolayers in vitro (HUVEC) to those of heme in solution: Radical oxygen species (ROS) production with several fluorescent probes; Apoptosis by FACS and fluorescent microscopy.

Results: Heme-loaded erythrocyte MP induced excessive ROS production, when contacting the endothelium. The effects were reproduced, in part, by synthetic MP artificially loaded with heme. These effects exceeded those of heme alone at similar concentrations. ROS production was inhibited by saturating MP PS with annexin-V, or by pre-treatment with hemopexin. Sickle cell MP differed from healthy MP in size, PS expression, and contained more heme and hemoglobin. ROS induced by sickle cell MP were released by HUVEC and not MP and production was blocked by pharmacological targeting of endothelial NADPH oxydase and PKC. In vitro, ROS induced endothelial apoptosis. The toxic effects of heme-loaded MP could be abrogated by hemopexin, a plasma protein that chelates free heme.

Discussion: We concluded that sickle cell MP are loaded with heme and stimulate endothelial ROS production through NADPH oxidase, and induce endothelial apoptosis in a more efficient fashion than heme alone. Our results show that erythrocyte degradation products can directly bridge hemolysis to vascular injury and could participate to vasculopathy in hemolytic syndromes like sickle cell disease.

0330

Erythrocyte Microparticles Can Induce Kidney Vaso-Occlusions in Sickle Cell Disease

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Background: Patients with sickle cell disease suffer from painful vaso-occlusive crises. These crises are associated with disseminated vaso-occlusions, raised levels of circulating erythrocyte microparticles as well as thrombospondin-1 (TSP1). Microparticules (MP) are submicron membrane vesicles shed by compromised or activated cells. We hypothesized that TSP1 mediates the shedding of erythrocyte MP and participates in vaso-occlusions.

Methods and Results: We injected TSP1 to transgenic SAD mice with sickle cell disease, sensitive to renal vaso-occlusions. We characterized circulating MP by FACS after phosphatidylserine labeling. TSP1 injection strongly stimulated MP levels (+260%) and initiated renal vaso-occlusions within 5 minutes, without impacting systemic parameters (heart rate, cardiac output). This was consistent with increased local vascular resistance. In vitro, TSP1 triggered a rapid conversion of purified erythrocytes into echinocytes carrying long and apparently fragile spicules. This was followed by MP shedding. These modifications of erythrocyte phenotype and MP shedding were recapitulated by peptides derived from TSP1 carboxyterminus. We purified the MP shed by erythrocytes in vitro, and administered them back into SAD mice. Erythrocyte MP triggered immediate renal vaso-occlusions, similar to those induced by TSP1. In vitro, purified erythrocyte MP triggered the production of radical oxygen species by endothelial monolayers, favored erythrocyte adhesion to them and induced endothelial apoptosis. Purified erythrocyte MP also compromised acetylcholine-dependent vasodilation in perfused microvessels. These effects of MP were inhibited by saturating surface phosphatidylserine with annexin-V, or with inhibitors of endothelial ROS production.

Conclusions: We conclude that TSP1 can trigger the production of microparticles by erythrocytes in sickle cell disease. Second, our data reveal that erythrocyte MP can induce endothelial injury, and this mechanism facilitates the occurrence of vaso-occlusive crises in mice. Therefore, our work supports a novel concept that toxic erythrocyte microparticles constitute a direct connection between sickle cell anemia and vascular disease.

0037

Microparticle release in remote ischemic conditioning mechanism

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Background: Remote ischemic conditioning (RCond) induced by short periods of ischemia and reperfusion of an organ or tissue before myocardial reperfusion is a powerful and attractive strategy of cardioprotection in the context of acute myocardial infarction. Nonetheless, its mechanism remains unknown. A humoral factor appears to be involved, although its identity is currently unknown. We hypothesized that the circulating microparticles (MPs) are the link between the remote tissue and the heart.

Methods: MPs from rats and healthy human undergoing RCond were characterized. In rats, RCond was induced by 10min of limb ischemia. In humans, RCond was induced by three cycles of 5min inflation and 5 min deflation of a blood-pressure cuff. In the second part of the study, rats underwent 40 min myocardial ischemia followed by 2 hours of reperfusion. Infarct size was measured and compared among three groups of rats: (1) myocardial infarction alone (MI) (n=6); (2) MI+RCond started 20min after coronary ligation (n=6); (3) MI+injection of RCond-derived rat MPs (MI+MPs) (n=5).

Results: MPs from endothelial cells (CD54+ and CD146+ for rats and humans, respectively) and procoagulant MPs (Annexin V+) markedly increased after RCond, both in rats and humans. RCond reduced infarct size (24.4±5.9% in MI+RCond *vs.* 54.6±4.7% in MI alone, $P<0.01$). Infarct size did not decrease in MI+MPs compared to MI alone (50.2±6.4% *vs*. 54.6±4.7%, not significantly different).

Conclusion: RCond increased endothelium-derived and procoagulant MPs in both rats and humans. However, MP release did not appear to be a biological vector of RCond in our model.